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Contribution of post-receptoral cells to the a-wave of the human photopic electroretinogram

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Abstract

ERGs were recorded to red flashes (0.01–50 ph cd s m^2) presented against a steady background (2000 sc td) or 0–300 ms after its suppression. The cone a-wave was altered in form and increased in amplitude in the dark. Peak amplitudes were doubled when the dark period was 50–100 ms and also when it was 150–200 ms. Measurement of the a-wave at fixed times showed that amplitude increase occurred at times later than 6–8 ms. The a-wave receives a significant negative-signal contribution from two post-receptoral mechanisms. These are adapted by weak backgrounds and recover their sensitivity extremely rapidly in the dark. The cone photocurrent alone contributes 40–70% of peak amplitude depending on stimulus intensity.

Keywords: Human ERG; A-wave; Cones; Adaptation

1. Introduction

It has generally been assumed that the leading edge of the human light adapted ERG a-wave is the direct reflection of currents from cone photoreceptors (Armington, 1974; Heynen & van Norren, 1985a, 1985b). However, early intra- and extra-cellular studies of vertebrate retinal signals indicated that the ERG a-wave may contain contributions from cells in other retinal layers (for a review see Armington, 1974). Recently it has been shown that application of PDA, which suppresses activity of inner retinal neurones, reduced the amplitude of the a-wave in macaque especially when the stimulus intensity was low (Bush & Sieving, 1994). This led to the conclusion that the primate electroretinogram, over the first log unit of intensity above photopic threshold, receives a significant negative-signal contribution from neurones post-synaptic to the cones; only for brighter stimuli did cone activity appear to contribute substantially to the photopic a-wave. Further studies in macaque showed that the cone a-wave consisted of a initial rapidly rising phase followed by a slower phase out to response peak. The second slower phase was enhanced in amplitude after a brief period of dark adaptation and was suppressed by a steady background and also by application of PDA (Robson, Saszik, Ahmed, & Frishman, 2003). This indicated that the later portion of the a-wave must be generated in inner retina.

These studies in macaque provide the most direct evidence for a post-receptoral contribution to the cone a-wave. In humans it is clear that the later part of both the rod (Robson & Frishman, 1998/1999) and cone (Hood & Birch, 1993, 1995) a-wave are obscured because of the intrusion of the positive b-wave and oscillatory potentials (OPs) so that the peak of the cone ERG a-wave occurs between 14 and 20 ms depending on stimulus intensity. However, recent studies in humans using cone transduction models (Paupoo, Mahroo, Friedburg, & Lamb, 2000; Smith & Lamb, 1997) have fitted only the first 9–15 ms of the response and other studies have interpreted only the first 11–12 ms of the response as directly reflecting cone photocurrents (Cideciyan & Jacobson, 1996; Hansen & Fulton, 2005; Hood & Birch, 1993, 1995) because of the possible contribution from post-receptoral cells at later

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times of the a-wave. One previous quantitative study in humans, using a paired flash procedure and a-wave modelling (Friedburg, Allen, Mason, & Lamb, 2004), showed that the later part of the a-wave was affected by the level of the background illumination and that the estimated size of the cone response could account for no more than 70% of the peak amplitude. They concluded that at times later than about 6 ms the a-wave contains a measurable contribution from post-receptoral sources.

Taken together, these studies suggest strongly that post-receptoral cells contribute to the human cone a-wave prior to intrusion of the b-wave. Their relative contribution may depend in part on the intensity of the stimulus and on the level of the background illumination. In order to provide further direct quantitative data we have employed a technique previously used in macaque in which a rod-suppressing background was briefly extinguished just before delivering the stimulus flash (Robson et al., 2003). We systematically varied the period of darkness and show that the later part of the human cone a-wave is also modified in form and amplitude and that the time course of the changes indicate contributions from at least one, but probably two cellular mechanisms likely to be post-receptoral to the cones. Measurement of the a-wave at various fixed times after the flash enabled a more quantitative estimate of the time at which these mechanisms begin to contribute to the response and this has also allowed us to estimate the proportion of the normal a-wave that is pure cone over a range of stimulus intensities.

Preliminary results were presented in abstract form at the annual meetings of the International Society for Clinical Electrophysiology of Vision (2005) and the Association for Research in Vision and Ophthalmology (2006).

2. Methods

2.1. Subjects

A total of 6 subjects (2 male, 4 female) aged 19–21 yr participated in the experiments. All subjects had normal visual acuity and colour vision (Ishihara test plates). Informed consent was obtained from each subject following detailed explanation of the purpose and nature of the ERG procedure.

2.2. Light stimulation

Adapting and stimulus lights were delivered in a custom-built integrating sphere (Ganzfeld). The adapting background light source was a 24 V, 250 W tungsten halogen lamp powered by a regulated DC supply. Light from the lamp was collected by an aspheric condensing lens, conditioned by heat- and ultra-violet absorbing filters and brought to a focus at the centre of a electronically operated shutter by a second aspheric lens. At the exit point of the shutter the light passed through a medium waveband blue gelatine filter (Kodak Wratten 47B) and was then delivered into the ganzfeld sphere by means of a four-tailed fibre optic bundle. The shutter was fully open within 6 ms from application of the trigger.

Long duration stimuli were produced using a second system identical to the one just described, except that in this channel the light finally passed through a red gelatine filter (Kodak Wratten 29). Short duration flash stimuli were produced by a xenon flash gun (Mecablitz 32 Z-2) powered by a stabilized DC supply. The flash gun was mounted at the top of the ganzfeld dome and out of direct view by the subject. The light was conditioned by heat and ultra-violet absorbing filters and then passed through a medium waveband red gelatine filter (Kodak Wratten 29) and speckled perspex diffuser before entering the ganzfeld through a small aperture. The electronic shutters and flash gun were controlled by commands from the data aquisition computer. Stimulus durations and intensities for each experiment are given in Section 3. The subject viewed the stimulus through a small viewing port and a camera with pinhole lens (3 mm) mounted opposite the viewing port allowed the experimenter to confirm that the subjects eye was fully open and still while the ERG was being recorded.

Light levels were measured using a calibrated photometer (International Light IL1700) fitted with either a scotopic or photopic filter. For measurement of the brief Mecablitz flashes the instrument was switched to integrating mode (5v reverse bias of the photodiode). Mecablitz flash stimulus intensity was controlled by varying the duration of the flash. Repeat measurements of individual flashes showed that the integrated output was reproducible to within ±5%. The flash gun was fully re-charged within 1 s.

2.3. Electrodes

Transient ERGs were recorded from one eye by means of a DTL electrode located in the lower conjunctival fornix. The reference electrode was located on the ipsilateral outer canthus and the ground electrode on the forehead. The eye was anaesthetised with Benoxinate 1% and the pupil dilated with Tropicamide 0.5%. The pupil diameter was between 8 and 8.5 mm for each subject. ERG electrodes were positioned under low level room illumination. When the pupil was fully dilated the room lighting was extinguished and the subject's eyes were adapted for a few minutes to a blue background of 39 sc cd m^2 (approximately 2000 sc td) before commencing the experiment.

Choice of reference electrode site proved difficult for recording ERG responses to long-flash stimuli. Preliminary experiments showed that these stimuli caused excessive blink or eye movement artefact with the result that the onset or even the whole period of the d-wave could be obscured or distorted. In an attempt to minimise this artefact several reference electrode positions were tried: disc reference electrodes were placed (i) on the skin at the ipsilateral canthus or (ii) on the lower eyelid of the contralateral eye; or (iii) a DTL electrode was placed on the cornea of the contralateral eye and that eye was covered. The optimum electrode configuration varied between subjects and we report the best available results.

2.4. ERG data acquisition

ERG data were acquired using custom software running on a laboratory PC. Signals were differentially amplified with a gain of 1000 over a bandwidth of 1–1000 Hz and digitized at a sample rate of 2 kHz with a resolution of 12 bits. The sweep time was 100 ms (transient responses) or 360 ms (long-flash responses). Eight to 16 responses were averaged for each trial depending on signal quality and a pre-set voltage window allowed automatic rejection of sweeps contaminated by artefact. At least two averaged responses were acquired for each test condition. The DC component, computed from the first pre-stimulus 10 ms, was subtracted from the response and a-wave amplitudes were measured relative to baseline from the off-line average of two or more independent trials. Peak-to-peak amplitude measurements were made for other components and baseline-to-peak measurements of the b-wave and Photopic Negative Response (PhNR) were also used as appropriate (see Section 3).

3. Results

3.1. ERG responses to a red stimulus presented against a rod-suppressing blue background

ERG responses were recorded in 3 normal volunteers (2 female, 1 male) to a red flash presented against a steady rod-suppressing blue background (39 sc cd s m^2, 2000 sc td).

There were 12 stimulus energies ranging from 0.01 to 50.0 ph cd s m^2 (flash duration 16–900 μs).

ERG responses of one subject are shown in Fig. 1; responses of the other 2 subjects were similar in waveform. The ERG response developed rapidly in waveform at low stimulus energies and all normal components could be identified to stimulus energies above 0.5 ph cd s m^2. At low stimulus energies the a-wave consisted of a single rising response but at the highest energies there was a clear discontinuity at 6–8 ms giving the appearance of a bifurcated response with a second limb following the initial fast rising phase (Fig. 1, right panel). The photopic negative response (PhNR) and i-wave were only clearly identifiable over a narrow range of stimulus intensities extending from about 0.1 to 6 ph cd s m^2. Fig. 2 shows amplitude response functions for all 3 subjects. A-wave peak amplitude increased steadily up to a stimulus intensity of 10 ph cd s m^2 after which there was no significant change. The number of oscillatory potentials (OPs) increased from 0 to 4 with increasing stimulus energy; averaging the first three OPs showed a maximal amplitude to stimulus intensities of 1–2 ph cd s m^2, comparable with that for the b-wave, PhNR and i-wave.

3.2. ERG responses to a red stimulus flash presented a short time after extinguishing the rod-suppressing background

Based on the previous results we next recorded ERGs to a 0.86 ph cd s m^2 red flash (duration 44 μs), which is sufficient to nearly saturate all ERG components except the a-wave, and to a 23 ph cd s m^2 flash (duration 348 μs) giving a bifucated and near maximal amplitude a-wave. Three subjects participated, two of whom had also taken part in Experiment 1. Responses were recorded initially to the red flash presented against a steady background and then to the same stimulus presented 0–300 ms after brief (100–400 ms) suppression of the background field. The subject's eye was exposed continuously to the rod-suppressing background between each experimental condition. ERG responses were also recorded to suppression of the background light (background OFF responses).

3.2.1. Blue background OFF ERG response

The OFF response (not shown) consisted of a small positivity with a maximum amplitude of about 15 μV beginning 20 ms after suppression of the background and lasting about 40 ms. This is small relative to the amplitude of the ON response and would cause minimal or no distortion of the a- and b-waves even when both stimuli were triggered simultaneously (see also next section). Therefore we have not made correction to ERG ON responses for any of the experimental conditions described below.

3.2.2. Changes to the A-wave

The red flash stimulus was delayed relative to background OFF in 25 ms incremental steps from 0 ms (stimulus flash occurred simultaneously with suppression of the

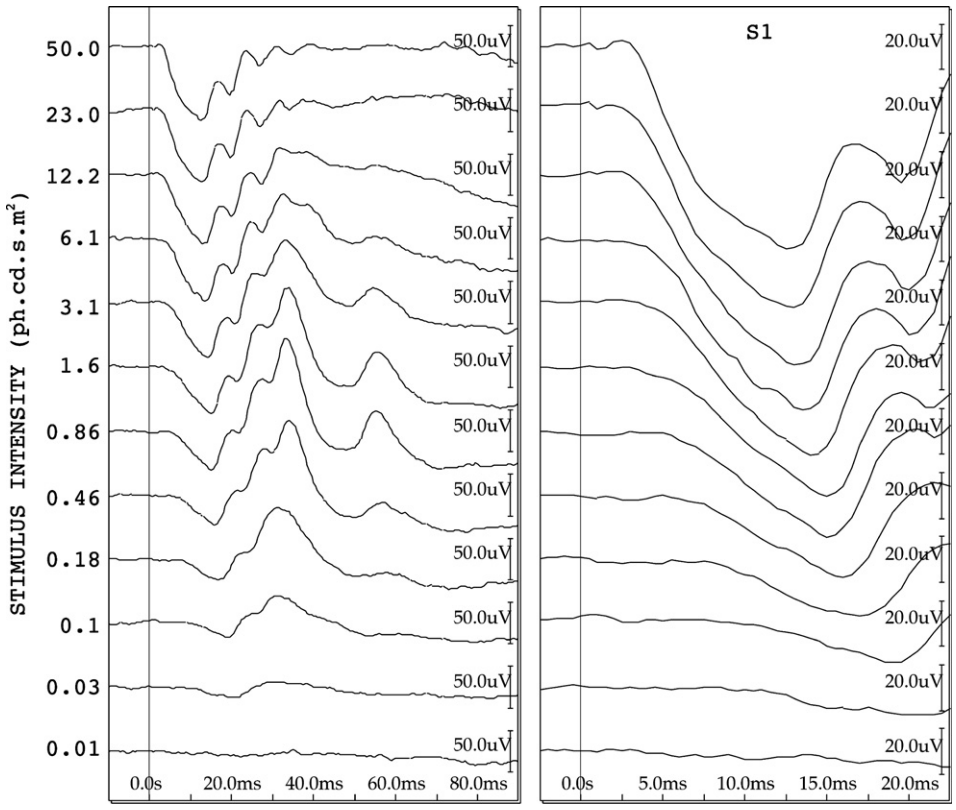

Fig. 1. Normal ERG responses of 1 subject to a red flash stimulus for a range of intensities from 0.01 to 50 ph cd s m^2 indicated to the left of each trace. Stimuli were delivered against a steady blue background. The right panel shows the a-wave on an expanded time scale.

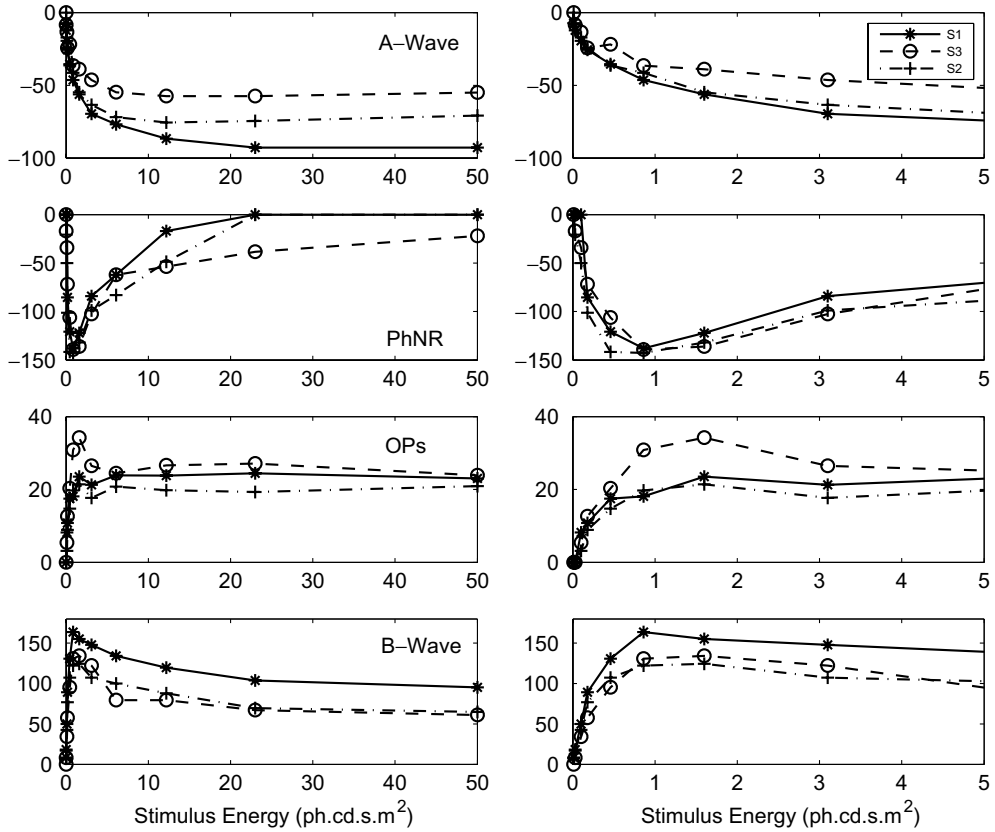

Fig. 2. Intensity-response functions for 3 normal subjects for each ERG component. Panel on the left shows response amplitudes over the full intensity range studied; panel on the right shows amplitudes for low intensity stimuli on an expanded scale.

background) to 200 ms and then by 250 and 300 ms. Fig. 3 shows a sub-set of ERG responses of one subject. When the stimulus flash occurred simultaneously with suppression of the blue background (0 ms delay, second trace down) ERG responses were comparable with responses recorded when the same stimulus was presented against a steady background (top trace). This confirms that the background OFF response had little effect on the ON response as discussed above.

Stimulus delays as short as 25 ms caused an apparent shift of 3 ms in the time to peak and longer delays caused a marked change to the form and amplitude of the a-wave. When the stimulus was delayed by 50–100 ms the a-wave to the low intensity stimulus (Fig. 3, left panel) had a second slower limb that was not evident in responses recorded in the presence of a steady background. The a-wave to the brighter stimulus was bifurcated even in the presence of a steady background, as already discussed, and delaying the stimulus by 50–100 ms accentuated the discontinuity between the initial rapid rise and the following slower rise to peak. A bifurcated form of a-wave was most evident when the stimulus was delayed by 50 ms at both flash intensities, as shown for all 3 subjects in Fig. 4. Responses recorded to the delayed stimulus were similar to those recorded in the presence of a steady background over the first 8–12 ms depending on stimulus intensity. Thereafter, the responses diverged with the response to the delayed stimulus showing a relative plateau out to a-wave peak and a greater amplitude response for all 3 subjects.

We measured the a-wave amplitude at a number of fixed times extending from 6 ms after the stimulus to the time of peak amplitude (about 15 ms) and results are shown in Figs. 5 and 6. Increasing the stimulus delay from 0 to 75 ms or 100 ms caused a increase in a-wave amplitude at all times of measurement and for both stimulus intensities. Amplitude decreased with longer delays and the maximal amplitude peak at 75–100 ms was sharply defined for all 3 subjects. For the more intense stimulus there appeared to be a second phase of amplitude increase with a further sharply defined amplitude peak associated with delays in the region of 150–200 ms (see especially subject S3, Fig. 6). Amplitude changes at early times (6, 8 and 10 ms) were small and transient. At later times (12, 14 ms and peak) the absolute amplitude increased much more and amplitudes remained significantly greater than those recorded in the presence of a steady background.

3.2.3. Changes to later components of the ERG

We now consider whether the brief period of darkness caused changes to other components of the cone-driven ERG. Fig. 7 (left panel) shows ERG response to a 0.86 ph cd s m^2 stimulus presented against a steady background and the superimposed response to the same stimulus presented from 0 to 100 ms after extinguishing the

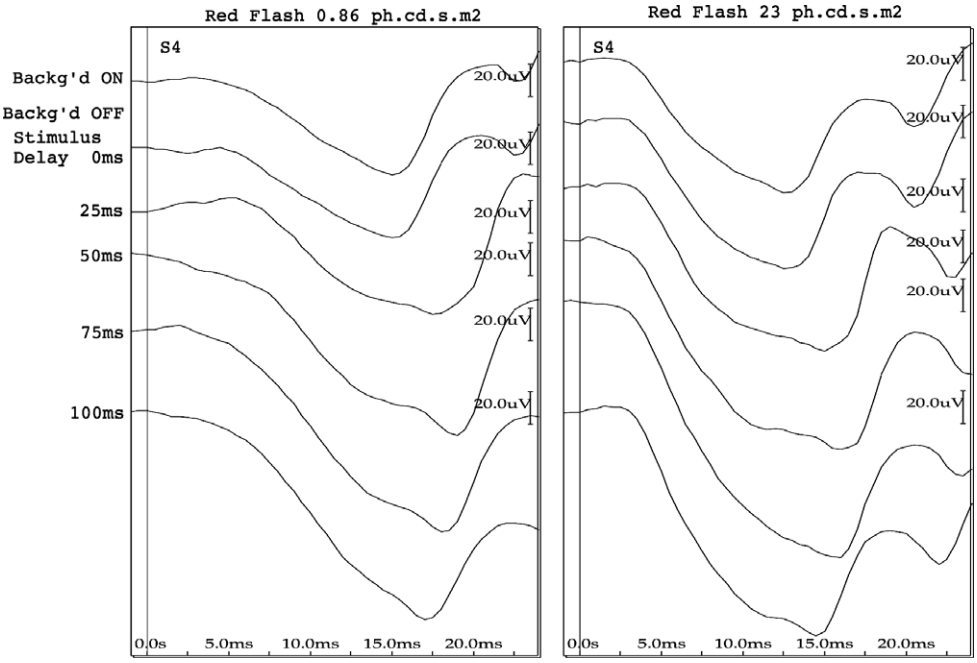

Fig. 3. ERG responses of 1 subject to a transient red flash of 0.86 (left panel) or 23 ph cd s m^2. The top traces were recorded in the presence of a steady blue background; the five remaining lower traces show responses recorded to the same red flash delivered 0–100 ms after extinguishing the steady blue background.

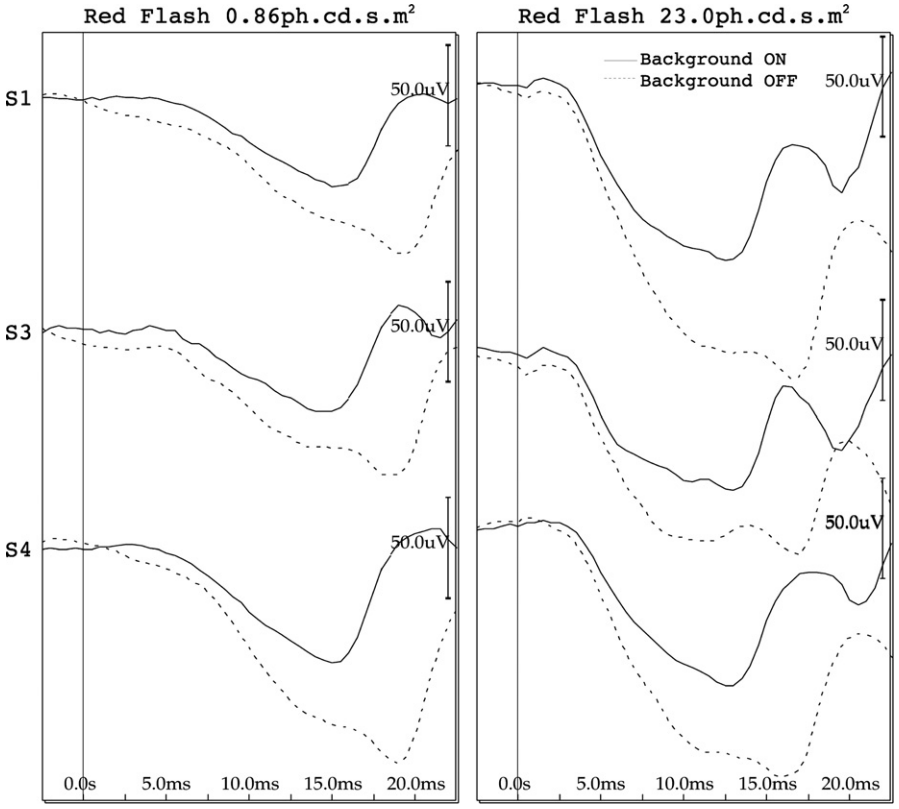

Fig. 4. ERG responses of 3 subjects to a 0.86 (left panel) or 23 ph cd s m^2 red flash. The flash was delivered against a steady blue background (solid line) or 50 ms after extinguishing the background (broken line).

background. Qualitatively the changes to later components appeared to follow directly from the change to the a-wave; peak latencies were initially prolonged by about 3 ms and the waveform was displaced progressively more negative with increasing stimulus delay. Peak-to-peak amplitudes were not altered significantly except for the PhNR which

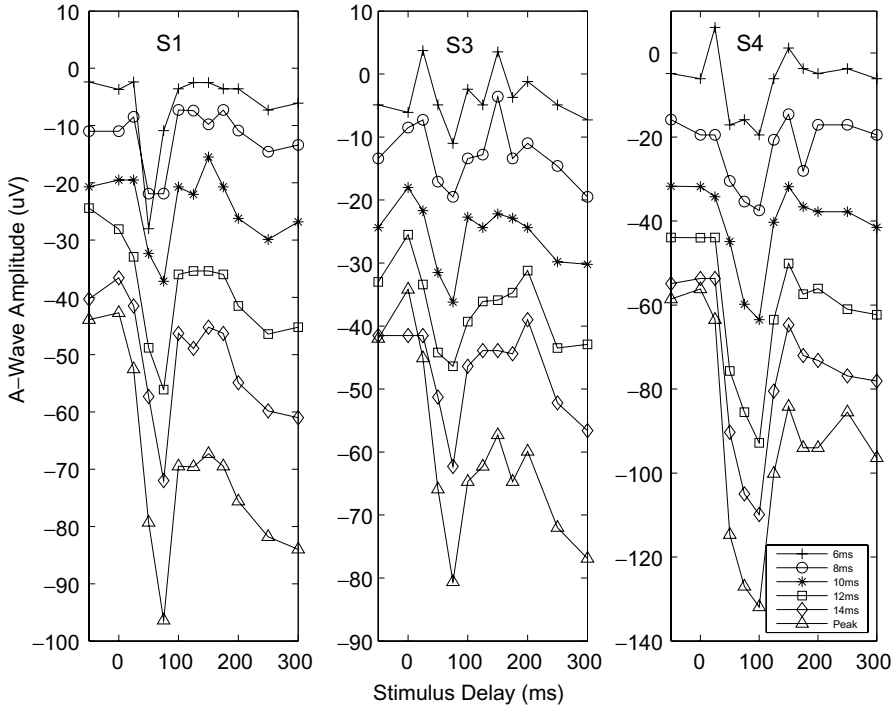

Fig. 5. Amplitude of the ERG A-wave for 3 subjects. The stimulus was a red flash of 0.86 ph cd s m^2. The stimulus was delivered against a steady background (shown as time −50 ms) and at various times after suppression of the background (shown as delay times from 0 to 300 ms). A-wave amplitude was measured at 6, 8, 10, 12, 14 ms and peak (traces plotted in order from top to bottom).

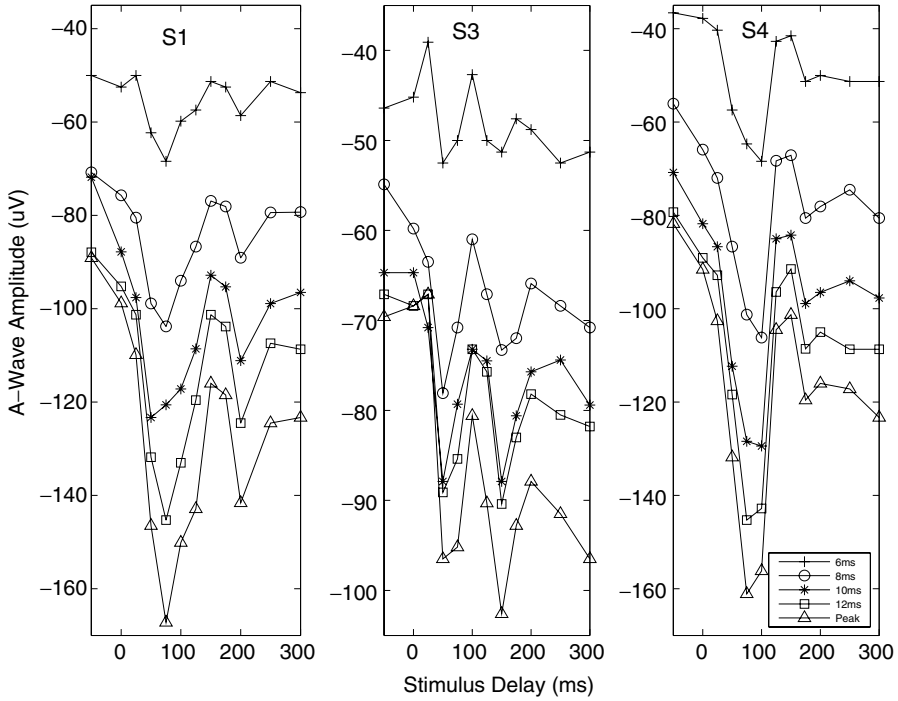

Fig. 6. As Fig. 5 but stimulus intensity was 23 ph cd s m^2.

was slightly reduced in all 3 subjects. Quantitative data shown in Fig. 8 confirm that the PhNR was reduced in amplitude for all three subjects by about 20% but the change was sustained over a wide range of stimulus delays. Peak-to-peak measurement of the b-wave did not change significantly for 2 subjects but increased in amplitude for 1 subject for stimulus delays of 75–150 ms. However, this may just be a reflection of the real increase in a-wave amplitude because baseline-to-peak measurements (not presented) showed a strong decrease in amplitude that was maximal when the stimulus was delayed by 75 ms (i.e. the amplitude changes mirrored those of the a-wave shown in

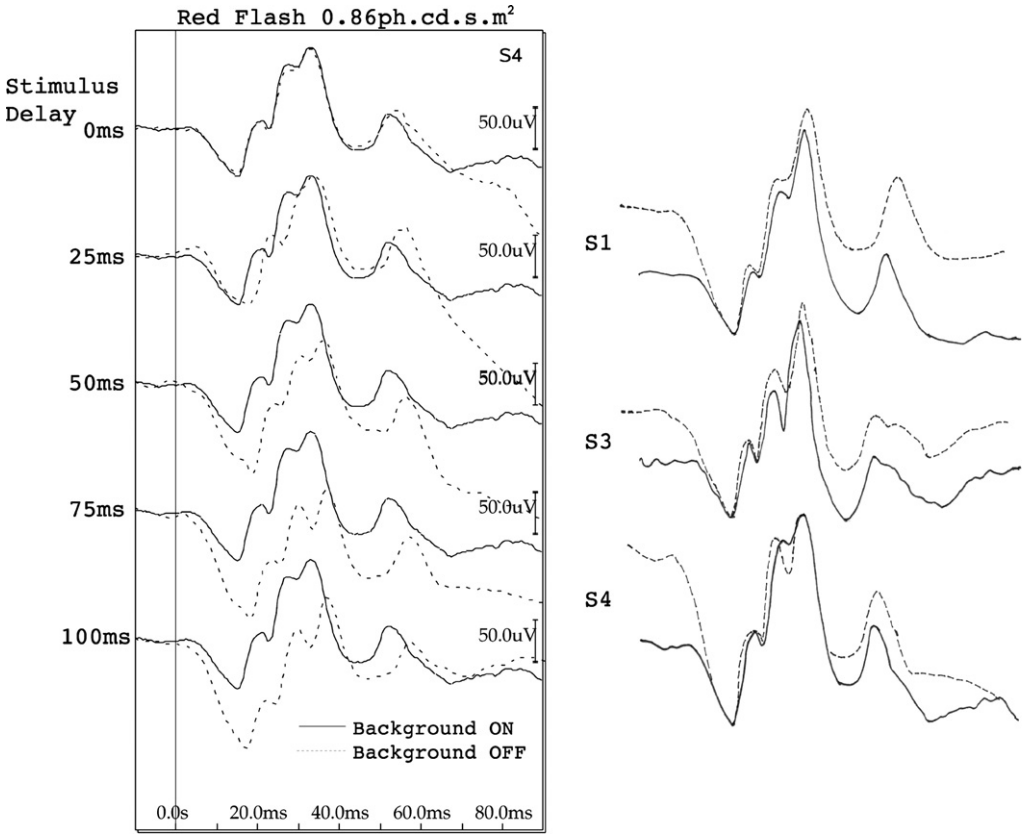

Fig. 7. Left panel shows ERG responses of one subject to a 0.86 ph cd s m^2 red flash presented against a steady blue background (solid line replicated down the figure) and responses to the same red flash delivered at times from 0 to 100 ms after extinguishing the background (broken line). Right panel shows responses of 3 subjects to the same stimulus presented against a steady background (solid line) and when delivered 75 ms after extinguishing the background (broken line). The responses to the delayed stimulus (broken line) have been displaced up and to the left by eye so that the peaks of the a-waves are aligned. See text for explanation.

Fig. 5). There was no systematic change in i-wave or OP amplitude. ERG responses to the brighter stimulus (not shown) showed similar changes.

3.3. ERG responses to a long-flash stimulus (ON–OFF ERGs)

Long-flash stimuli were used next to enable identification of OFF as well as ON responses of the ERG. We specifically considered whether the OFF (d-wave) response of the ERG was affected by brief suppression of the same adapting field.

ERG responses were recorded to a red flash (KW 29) with a stimulus duration of 200 ms and an intensity of 34 ph cd m^2. The stimulus delay relative to background OFF was changed with the same step sequence as used in Experiment 2. The background was extinguished for a period varying from 375 to 675 ms and the repetition period was lengthened to 5 s because of the longer stimulus duration and the presence of both ON and OFF components in the response. All the subjects tested found this stimulation sequence visually uncomfortable and caused them to blink excessively. In consequence, either the start or the whole time course of the d-wave was often obscured by eye-blink generated artefact. A variety of reference electrode positions were tried in an attempt to minimise this artefact but none gave completely artefact-free responses over the full range of stimulus delay settings. To date we have not been able to construct reliable d-wave amplitude plots of the form shown in Fig. 5. Therefore, we report the responses obtained with the electrode configuration that gave the technically best ERGs for stimulus delays from 0 to 100 ms.

Fig. 9 shows the a-wave and d-wave responses of one subject on an expanded time-base. The later part of the a-wave increased in amplitude with increasing stimulus delay in a way similar to that observed for the transient flash ERG response (Figs. 3 and 4). However, the d-wave showed no such systematic change and certainly not to the rising phase of the response; there were minor differences to the later negative-going phase which may be residual noise. The responses of one other subject (not shown) were similar; technically satisfactory d-wave responses could not be obtained from two other subjects.

3.4. Re-consideration of a-wave amplitude changes related to stimulus intensity

We tentatively conclude that the observed alteration of the later part of the a-wave may be due to a contribution from cells proximal to the cones. With this in mind we have

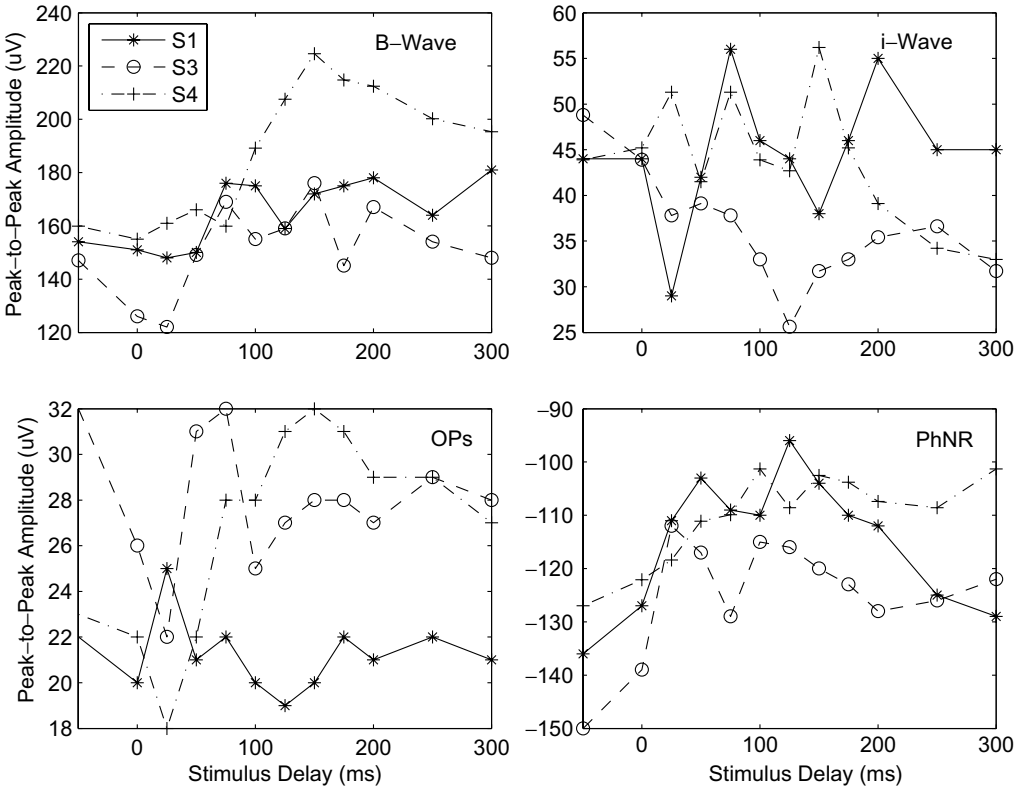

Fig. 8. Peak-to-peak amplitude plots of inner retinal ERG components for 3 normal subjects. The stimulus was presented against a steady background (shown as time −50 ms) and at various times after suppression of the background (shown as delay times from 0 to 300 ms).

re-examined the data from Experiment 1. As an approximation we have taken 8 ms as the latest time in the a-wave that is due to cone photocurrent alone. We have measured the amplitude of the a-wave at 8 ms and expressed it as a percentage of a-wave peak amplitude for each stimulus intensity. Fig. 10 shows that at low stimulus energies (2–4 ph cd s m^2) the amplitude of the a-wave at 8 ms is less than half of the a-wave peak amplitude; this rises to a maximal contribution of 70–80% to stimulus intensities between 20 and 25 ph cd s m^2.

4. Discussion

Our main finding is that the human cone a-wave was altered in form and increased in amplitude when the stimulus was preceded by a brief period of darkness, compared with the response to the same stimulus presented against a steady background. Could the cone or rod photoreceptors contribute to these changes? We think it is unlikely that they are due to recovery of rod signals in the dark. Our blue adapting light delivered 39 sc cd m^2 (approximately 2000 sc td) and the subject's eye was exposed to this light level continuously except for the brief (a few hundred milliseconds) dark period surrounding delivery of the red stimulus flash. Previous ERG studies indicate that this procedure would completely suppress the rod circulating current for the full duration of the experiment (Friedburg, Thomas, & Lamb, 2001; Hood & Birch, 1993; Paupoo et al., 2000; Robson et al., 2003). These previous studies also suggest that our background light level is unlikely to affect the cones significantly and this is supported by three further lines of evidence. Firstly, much brighter backgrounds (3100 sc cd m^2) that bleach about 90% of cone photopigment reduced the a-wave by no more than 50% (Kenkre, Moran, Lamb, & Mahroo, 2005). Secondly, our background light level of 39 sc cd m^2, equating to 4.05 ph cd m^2 (approximately 200 ph td), was 10 times less intense than that required to halve the flash sensitivity of a single monkey cone (Schnapf, Nunn, Meister, & Baylor, 1990; Schneeweis & Schnapf, 1999). Thirdly the modification in the form of the a-wave is independent of stimulus energy (see later), unlike the behaviour of photoreceptors themselves.

Previous studies of the human cone-driven ERG recorded with a steady rod-suppressing background show a bifurcated a-wave similar to the responses we recorded to more intense stimuli (Friedburg et al., 2004; Hood & Birch, 1993, 1995; Paupoo et al., 2000). It was also shown that the later second limb could be progressively suppressed by increasing the intensity of a steady background and that the derived cone receptor response followed the a-wave at early times but was smaller than the a-wave at times later than 8 ms (Friedburg et al., 2004; Paupoo et al., 2000). We have shown that the a-wave to a low as well as a high energy stimulus is bifurcated following a short period of dark adaptation. We quantified the

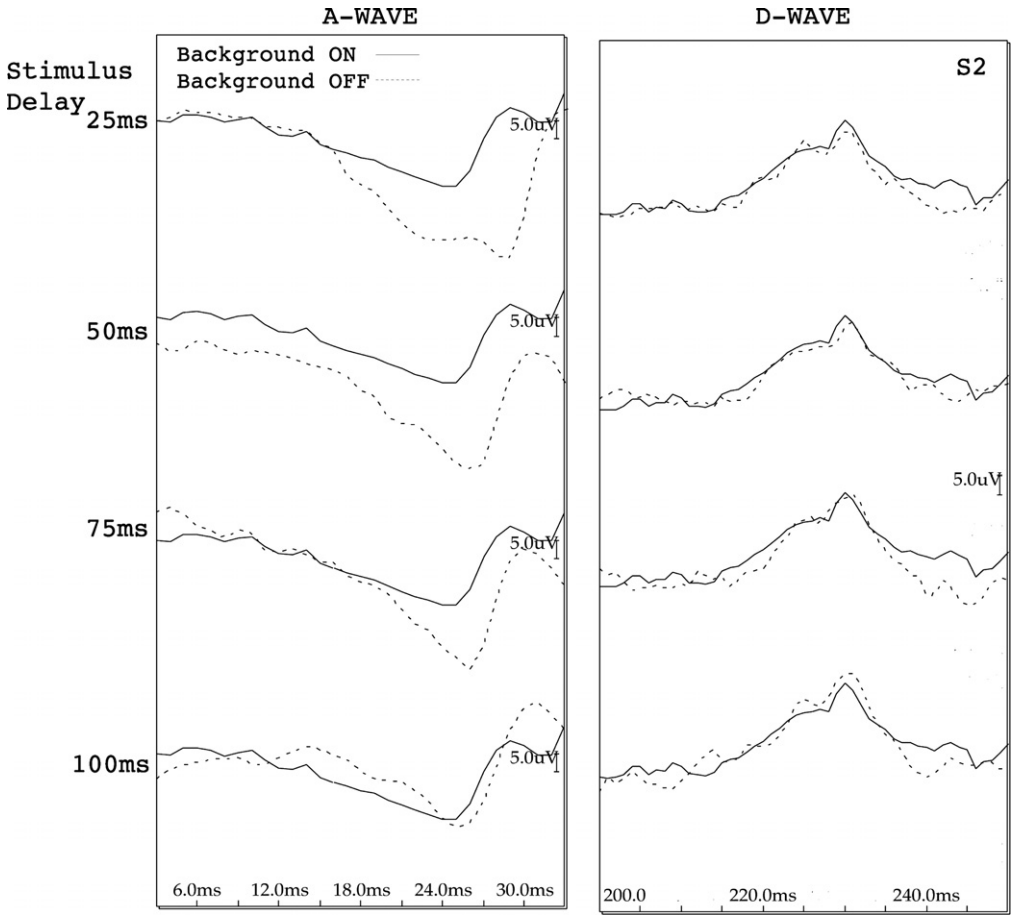

Fig. 9. Long-flash ERG A-wave (left panel) and D-Wave (right panel) responses of 1 normal subject. Responses were recorded to a 200 ms red flash presented against a steady blue background (solid line replicated down the figure) and responses to the same red flash delivered at times from 25 to 100 ms after extinguishing the background (broken line).

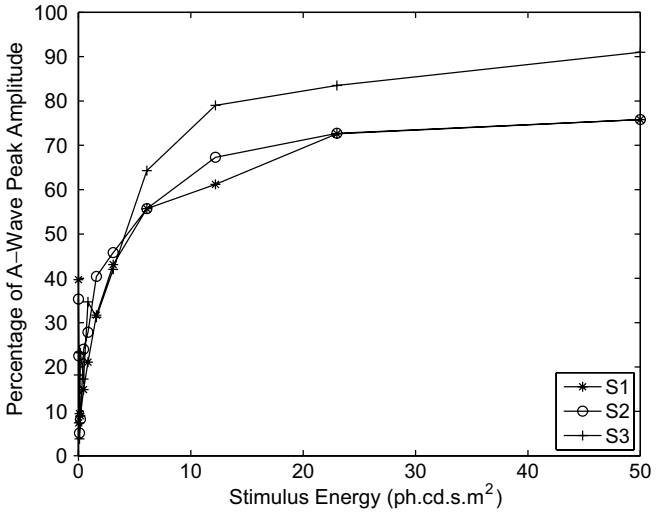

Fig. 10. ERG A-wave amplitude measured at 8 ms and expressed as a percentage of the amplitude at a-wave peak. Stimuli were red flashes from 0.01 to 50 ph cd s m^2 presented against a steady blue background. Data from Experiment 1; see text for details.

amplitude increase in the dark by measuring the a-wave at a number of fixed times from 6 ms to peak (at about 15 ms), for a range of dark adaptation durations. Amplitude increased only slightly at early times of measurement (6–8 ms) and the increase was transient. At later times of measurement amplitude increase was greater and more sustained; maximal peak amplitude was more than double that recorded with the steady background and remained 1.5–1.9 times larger when the dark period was 300 ms. These results add further quantitative evidence that the later part of the human a-wave can be modified independently of the early part of the response, that the changes are dependent on the level of background illumination and are qualitatively similar for low and high energy stimuli.

A response with two distinct parts strongly suggests that there must be contributions from more than one cellular process or mechanism. How many contributory sources are there? We systematically varied the length of the dark period in order to study the time course of the changes and found that there was a sharply-defined maximal a-wave amplitude associated with dark periods of 75–100 ms for both low and high energy stimuli (see Figs. 5 and 6) and a second shaply-defined peak at 150–200 ms for the high intensity stimulus. These findings imply at least one and probably two contributory mechanisms. Moreover, these changes to low intensity stimuli were

qualitatively similar to those described in non-human primate. Robson et al. (Robson et al., 2003) showed that the macaque cone a-wave consisted of a initial rapid rise followed by a slower phase out to response peak. This second limb was enhanced in amplitude when the background was extinguished 100 ms before the stimulus. It could also be suppressed by application of PDA, indicating that this later portion must be generated in inner retina. Given that the form of the macaque and human a-wave are similar (Robson et al., 2003) we can summarise our conclusions so far, as follows. The change in the form and amplitude at later times of the a-wave are due to intrusion of negative-polarity signals that sum with the cone photocurrent. The cellular mechanisms involved can be adapted more easily than the cone photocurrent, they can be adapted by relatively weak adapting fields and they recover their sensitivity extremely rapidly in the dark. There are probably two sources post-synaptic to the cones and they have different time-constants of recovery in the dark.

The ERG contains a number of signals of negative polarity (Niemeyer, 2005) but it is not clear what cells or mechanisms generate this late portion of the a-wave. Previous studies in non-human primate showed that the ERG a-wave could be modified by administration of various pharmacological agents that block the activity of inner retinal neurones but do not affect the photoreceptors (Bush & Sieving, 1994; Evers & Gouras, 1986; Falk & Shiells, 1986; Rangaswamy et al., 2004). These studies indicated that horizontal cells (HC), hyperpolarising bipolar cells (HBC) or more proximal cells in the OFF pathway sum with the photoreceptor response (Bush & Sieving, 1994; Evers & Gouras, 1986; Robson et al., 2003), but HBC were considered the more likely candidate (Bush & Sieving, 1994; Robson et al., 2003). Therefore, we investigated whether ERG components generated by bipolar and more proximal cells were also altered by suppression of the background light. The peak-to-peak amplitude of the b-wave, OPs and the i-wave did not change systematically with stimulus delay. The amplitude of the d-wave of the long-flash ERG response also did not change but our limited data do not allow us to conclusively exclude this possibility. The PhNR (Viswanathan, Frishman, Robson, Harwerth, & Smith, 1999) did decreased in peak-to-peak amplitude but the overall amplitude change was relatively small (20%) and sustained over a longer time period so it seems unlikely that a single cellular mechanism can account fully for changes to both the a-wave and PhNR. These findings imply that the increase in a-wave amplitude was not caused by the same cellular mechanisms that underly the generation of the normal ON or OFF inner retinal components of the ERG.

It is equally possible that the changes are generated at least in part in more distal retina. Falk and Shiells (Falk & Shiells, 1986) provided direct evidence for a HC contribution by showing that a negative polarity signal remaining after APB had a time course similar to that of HC cells. It has also been shown that the late afterpotential of Type-A HC in rabbit occurs concurrently with the PIII negative component of the ERG over a range of stimulus intensities, implying that the initial potential of HCs also contribute to the beginning of the ERG but are obsured by the b-wave (Hanitzsch, Karbaum, & Lichtenberg, 1999). Rabbit HCs also dark adapt extremely quickly and response amplitudes are much larger in the dark adapted than in the light adapted eye over a range of intensities (Hanitzch personal communication of unpublished data). A contribution from cells in distal retina could offer an explanation for the observation that the earliest change in the a-wave was a delay peak latency of approximately 3 ms. We speculate that this time period may be sufficient to quickly and selectively release these distal mechanisms from light suppression and their initial contribution to the a-wave is manifested as a relative plateau which extends the time to peak. Since the b-wave, OPs and PhNR are generated more proximally in inner retina (Viswanathan et al., 1999), these response peaks are also delayed by 3 ms relative to the response recorded against a steady background (see Fig. 7).

Although Hood and Birch (Hood & Birch, 1993) demonstrated that the first 10–15 ms of the human cone a-wave changed in amplitude with flash energy in ways generally consistent with responses from single cones, they and subsequent studies have taken care to restrict analysis to the first 11–12 ms of the a-wave, rather than take the peak at about 15 ms, to minimise the effect of contamination cause by intrusion of the positive b-wave and OPs (Cideciyan & Jacobson, 1996; Hansen & Fulton, 2005; Hood & Birch, 1993, 1995). The most direct quantitative data for a negative-signal contribution to the human cone a-wave was provided by Paupoo et al. (Paupoo et al., 2000). They showed that the a-wave response to an intense flash had a relatively flat plateau after about 6 ms in the presence of a intense background but continued to rise beyond 6 ms when the background was dim, and so restricted the fit of their transduction model to the relatively early time of 6 ms (Friedburg et al., 2004; Paupoo et al., 2000). Parsing the a-wave into receptoral and post-receptoral components is clearly difficult and the relative contribution of each may well vary depending on stimulus intensity. The parametric studies we described provide further quantitative data to help refine the range of possibilities: (1) the a-wave was bifurcated to the highest intensity stimuli presented against a steady background; the point of transition between the initial rapid rise and the slower phase occured at about 7 ms. (2) When the background was suppressed the a-wave to a low intensity stimulus changed to a bifurcated form with a point of transition at about 12 ms. (3) Dark and light adapted responses were similar only over the first 10–12 ms to low intensity stimuli and only over the first 5–6 ms to more intense stimuli. (4) Dark adaptation caused only transient and small amplitude changes at 6 and 8 ms but a greater and more sustained increase at later times. Based on these observations we speculate that post-receptoral cells contribute to the a-wave at times beginning no

later than 10 ms to low intensity stimuli and probably as early as 6 ms at higher intensities.

Given this strong evidence for a post-receptoral contribution we considered how much of the peak a-wave amplitude could be accounted for by the photoreceptor current alone. Taking 8 ms as a approximate cut-off point we found that for stimulus intensities up to about 3 ph cd s m^2, which is well below the level required to obtain a saturated a-wave, only about 40% of the total a-wave amplitude was accounted for by the photoreceptor component. The photoreceptor contribution rose to a maximum of 70–80% for stimulus energies over 20 ph cd s m^2, consistent with a previous report (Friedburg et al., 2004). These and previous results (Bush & Sieving, 1994; Friedburg et al., 2004) have implications for recording the clinical ERG because current guidelines (Marmor, Holder, Seeliger, & Yamamoto, 2004) recommend measurement of peak amplitude to a stimulus of intensity 1.5–3 ph cd s m^2. Over this intensity range it would be prudent to take only the first 8 ms, or 40% of peak amplitude of the a-wave, as directly reflecting the cone photoreceptor response (see Fig. 10). It is clear that considerable care must be exercised in interpreting a abnormally low peak amplitude.

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